

Evaluation of a metofluthrin fan vaporizer device against phlebotomine sand flies (Diptera: Psychodidae) in a cutaneous leishmaniasis focus in the Judean Desert, Israel

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ABSTRACT: The OFF! Clip-On fan vaporizer device releasing metofluthrin was evaluated against phlebotomine sand flies in the Judean Desert, Israel, in October, 2009. A total of 76,400 sand flies was collected, with male flies representing 98.3% *Phlebotomus sergenti* and 1.7% *P. papatasi*. Females comprised 43.0% of the total catch and included 6.7% blood-fed females. Similar proportions of flies were collected in both suction and sticky traps. In trials with unbaited suction traps, similar numbers of sand flies were collected in traps with a metofluthrin device, blank device, or no device (i.e., suction only). In suction traps baited with CO₂, higher numbers of *P. sergenti* males and blood-fed females were collected in traps with a blank device compared to traps with a metofluthrin device. In sticky traps baited with CO₂, there were no significant differences between catches in traps with a metofluthrin device, blank device, or no device. The results suggest metofluthrin from the device is not repellent against sand flies in a field environment despite showing insecticidal activity against flies collected in suction traps. *Journal of Vector Ecology* 36 (Supplement 1): S157-S165. 2011.

Keyword Index: Sand fly, *Phlebotomus*, spatial repellent, fan vaporizer, metofluthrin.

INTRODUCTION

Leishmaniasis is a serious global public health problem and a current operational military threat to U.S. and Allied military forces deployed in the Middle East and southwestern Asia. *Leishmania* parasites are transmitted by phlebotomine sand flies (Diptera: Psychodidae). Currently there is no vaccine or prophylaxis available to prevent parasite transmission, so vector control is crucial to prevent bites from potentially infectious sand flies. In the Judean Desert in Israel, *L. tropica* is vectored by *Phlebotomus* (*Paraphlebotomus*) *sergenti* sand flies that live on rocky hillsides and travel to residential areas at night in search of a blood meal. Local *P. sergenti* flies are exophilic (Schnur et al. 2004, Orshan et al. 2010) and highly abundant in outdoor collections from May until October (Orshan et al. 2010). During 2004-2005, 127 cases of cutaneous leishmaniasis (CL) caused by *L. tropica* were reported in the town of Ma'ale Adummim in the Judean Hills (population approximately 33,000) (Singer et al. 2008). No cases of *L. major* were reported, though the vector (*P. papatasi*) is a nuisance pest in the area (Schnur et al. 2004, Orshan et al. 2010). Conventional methods for outdoor sand fly control (e.g., residual and ULV spraying) are not only expensive and harmful to the environment, but they have also been proven ineffective in harsh desert conditions (Coleman et al. 2006, Orshan et al. 2006). The inconvenience posed by the nightly use of topical repellents has facilitated the search for alternative vector control methods that protect people against sand fly bites.

The recent development of novel synthetic pyrethroid insecticides with high vapor action at ambient temperature

has led to the development of passive control devices that aim to control flying insects without the application of heat (Ujihara et al. 2004). Metofluthrin is a particularly potent vapor active, pyrethroid with strong bite inhibition and knockdown activity against mosquitoes (Ujihara et al. 2004, Ishiwatari et al. 2009). Paper or plastic strips, resin emanators, and coils impregnated with metofluthrin have demonstrated spatial repellency against mosquitoes (*Culex*, *Aedes*, and *Anopheles* spp.) in the laboratory (Argueta 2004a, Kawada et al. 2004a, Lucas et al. 2007), indoor domestic environments (Kawada et al. 2004a, 2005b, 2006), and various outdoor environments (Argueta 2004b, Kawada et al. 2004b, 2005a, Lucas et al. 2007, Shono et al. 2004) based on light trap catches or human landing counts. Lukwa and Chiwade (2008) observed that smoke from metofluthrin-impregnated mosquito coils was repellent to *An. gambiae* mosquitoes in experimental huts for up to five h. However, Rapley et al. (2009) did not observe spatial repellency of impregnated paper emanators against *Ae. aegypti* mosquitoes in a controlled domestic setting despite a reduction in mosquito biting rates and increased mortality rates.

Fan vaporizer devices that emanate metofluthrin are marketed as potentially promising tools with long-lasting, spatial protection against mosquitoes. In the U.S. commercial market, the OFF! Clip-On mosquito repellent (S.C. Johnson & Son, Racine, WI) is a device that can be worn at waist level and houses a battery-powered fan to disperse metofluthrin at a continuous release rate for up to 12 h. To date, only one study describing the evaluation of a fan vaporizer device has been published: a device containing 0.6% metofluthrin (Fumakilla, Tokyo, Japan) provided 50-

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60% bite protection against *Ae. albopictus* mosquitoes on different areas of volunteers in semi-field studies in very still winds (Lee 2007). However, there are no published studies describing the efficacy of a metofluthrin fan vaporizer against phlebotomine sand flies. The main goal of this study was to evaluate the spatial repellency of the OFF! Clip-On device fan vaporizer device (31.2% w/v metofluthrin a.i.) against phlebotomine sand flies in a CL focus in the Judean Desert, Israel.

MATERIALS AND METHODS

Study area

The study was conducted at the edge of a perimeter road at the top of a barren hill near the village of K'far Adummim on the eastern slopes of the Judean Hills (population approximately 2,500). The study area is characterized by rocky slopes and gorges whose crevices are home to small mammals including the rock hyrax (*Procavia capensis*), a reservoir host of *L. tropica* (Jacobson et al. 2003, Svobodova et al. 2006, Talmi-Frank et al. 2010). Densities of sand flies measured by trapping collections have increased significantly since records began in 2005 (Orshan et al. 2010). In the present study, trials with suction traps were conducted on four nights (5-7 and 21 October, 2009), and trials with sticky traps were conducted on two nights (7 and 27 October, 2009). On each night of sampling, traps were arranged in a linear fashion along the perimeter road and operated for 11.5 h from sunset until sunrise (18:00 to 05:30).

Metofluthrin emanator device

The OFF! Clip-On Mosquito Repellent (S.C. Johnson & Son, Racine, WI) is a fan vaporizer device (henceforth termed "device") registered by the U.S. EPA in 2007. The device consists of a blue, hard plastic case that contains a cartridge impregnated with metofluthrin (31.2% w/v) and two AA batteries to operate the fan (Figure 1a). Metofluthrin volatilizes at ambient temperature, so heating is not required. An on-off switch can be toggled to turn on the fan, resulting in the suction of air into the top of the device, and the flow of air over the impregnated substrate in the cartridge causes the release of metofluthrin through a grill of holes located on the front of the device. It is estimated the evolution rate assessment is approximately 5-10 mg a.i. per hour at a continuous release rate. The manufacturer packaging claims the device provides protection against mosquitoes for 12 h. The device has a clip that allows it to be worn on the waist or clipped onto a clothing accessory.

Climate conditions

Mean measurements of temperature, relative humidity, wind speed, and direction were recorded at a height of 2 m every 10 min by the Israel Meteorological Service (IMS) station in Ma'ale Adummim, located approximately 6 km away. In addition, mean temperature, wind speed, and direction were recorded at ground level for 10 s every 60 s using a HOBO[®] weather station (Onset Computer



Figure 1. (a) OFF! Clip-On fan vaporizer device opened to show the metofluthrin cartridge and (b) dead sand flies on top of the cartridge. The device was operated by a battery-powered fan located under the cartridge (not shown).

Corporation, Bourne, MA) at another location in Ma'ale Adummim.

Suction trap experiments

The device was evaluated using CDC-type light traps (i.e., suction traps) modified to operate on two 1.5V GP2700 rechargeable AA batteries and positioned in an updraft configuration without light (Orshan et al. 2010). Each trap was held in a vertical position by a tripod, and the trap opening was approximately 10 cm above the ground (Figure 2). Six treatments were compared to test the comparative repellency of metofluthrin when CO₂ was present or absent. In three of the six treatments, traps were baited with approximately 1 kg dry ice (CO₂) contained in a thermos flask and vented at the trap entrance, and the traps were also paired with a metofluthrin device containing a metofluthrin cartridge (termed "CO₂ + MET"), a blank device containing no metofluthrin cartridge ("CO₂ + Blank") or no device ("CO₂ only"). In the other three treatments, traps were unbaited (i.e., no CO₂) and paired with a device containing metofluthrin ("MET"), a blank device containing no metofluthrin cartridge ("Blank") or no device ("Trap only"). Metofluthrin cartridges and batteries were replaced in traps each night. A total of 39 trials was conducted in which treatments were replicated three, six, or nine times (Table 1).

In all trials with a device, the fan was switched on regardless of whether the device contained a metofluthrin cartridge or no cartridge, resulting in the emanation of metofluthrin or air from the device, respectively. The device was either (i) placed in a horizontal position under the trap entrance, with the device fan facing upwards and emanating metofluthrin (or air from a blank device) in the general downwind direction (5-7 October only) (Figure 2), or (ii) placed in a vertical position and clipped onto the body of the trap facing downwind, with the metofluthrin (or air) emanating towards the ground (21 October only). Traps were placed approximately 3-5 m apart, and each tripod holding a trap was fixed in position using large stones attached to the tripod legs with elastic bands to prevent traps from being blown over in the wind.

Table 1. Experimental design for experiments with suction and/or sticky traps. Letters represent the order in which suction traps (A-D) and sticky traps (a-c) were arranged each day, with letters denoting the order in which treatments were placed. The total number of trials for each treatment is shown in the far right column.

Trap Type	Treatment	5 Oct	6 Oct	7 Oct	21 Oct	27 Oct	Total
Suction trap	Trap only			D	A		6
	Blank device				C		3
	MET			C	B		6
	CO ₂ only	C	B	B			9
	CO ₂ + Blank	B	A				6
	CO ₂ + MET	A	C	A			9
Sticky trap	Trap only			a			3
	CO ₂ only			b			3
	CO ₂ only				a		3
	CO ₂ + Blank				b		3
	CO ₂ + MET				c		3

Sticky trap experiments

The efficacy of the device was also evaluated using sticky traps baited with or without CO₂ to determine the effect of metofluthrin on the true repellency of sand flies collected passively instead of by active trap suction. Each sticky trap consisted of a sheet of white Mapalon polypropylene sheeting (ISO size A1, 70 cm x 100 cm, Mapal Plastics Mevo Hamma, Israel) coated with castor oil using a roll mop to achieve an even coating. The oiled sheets were placed on the ground approximately 2-3 m apart, and the corners of each sheet were weighted with large stones to keep the sheets fixed in the wind (Figure 3). For trials with CO₂, tubing connected to a thermos containing approximately 1 kg dry ice was inserted through a "+" incision at the center of each sheet, and the thermos was placed next to the southwest corner of each sheet. On the first night of sampling, two treatments were compared to determine the feasibility of using sticky sheets as passive traps to collect sand flies: three sheets were baited with CO₂ ("CO₂ only"), and three sheets were unbaited ("Trap only"). On the second night, the efficacy of the metofluthrin device was evaluated in three treatments with three sheets per treatment: sheets baited with CO₂ and paired with a device ("CO₂ + MET"), a blank device ("CO₂ + Blank"), or no device ("CO₂ only"). A total of 15 trials was conducted using sticky traps (Table 1).

Measuring sand fly population densities

For trials without CO₂, all male and female sand flies were counted in each suction and sticky trap collection. For suction trap collections with CO₂, due to the very large numbers collected, the total number of male and female sand flies in each trap collection was estimated by extrapolating the weight of 150-200 flies in a sub-sample relative to the weight of all the flies in the total trap collection. For sticky

trap collections on the first night of sampling, the total number of male and female sand flies was estimated by extrapolating a known number of flies in 1/8 of the total sheet area (approximately 10 cm x 10 cm) near the center of the sheet. For sticky trap collections on 27 October, the total number of sand flies was counted on each sheet; i.e., female or blood-fed flies were not recorded separately. The species composition was estimated for male sand flies in the total collection, or in the sub-sample of flies from CO₂-baited traps, by identifying the male flies to species. The absolute number of blood-fed females was recorded for each suction trap collection and for sticky trap collections (7 October only). For collections from CO₂-baited suction traps, blood-fed females were removed from the sub-sample of flies prior to estimating the total number to prevent over-estimating the weight of the total sample and hence the total number of flies.

Statistical analysis

Statistical analysis was performed using the SPSS 14 software package (SPSS 2005). Male *P. papatasi*, male *P. sergenti*, unfed females, blood-fed females, and all females were analyzed separately. Catches of flies (n) were transformed to $\log_{10}(n+1)$ and proportions (p) transformed to $\ln[p/(1-p)]$ (logit) to correct skewed error distributions and meet the assumptions of parametric tests. The transformed data were subjected to a one-way analysis of variance (ANOVA; F-test) to assess the impact of the factors TREATMENT, DAY, and LOCATION on the variance in the catch data. When ANOVA was significant, a post-hoc analysis was performed using Tukey's honestly significant difference (HSD) test (equal variances assumed) or Tamhane's T2 test (equal variances not assumed) based on whether Levene's Test of Homogeneity of Variances was

significant. Mean catches were compared using a 2-tailed Student's t-test for independent samples. Significance was assessed at $P < 0.05$.

RESULTS

Suction trap experiments

Approximately 56,000 flies were collected in 39 suction traps on four nights of trapping in October 2009. Female flies comprised 43.3% of the overall catch, and 5.9% of the females contained blood meals (one fly contained two blood meals) (Table 2). Male flies consisted of 98.3% *P. sergenti* and 1.7% *P. papatasi*. Higher numbers of flies were collected in suction traps baited with CO_2 (range = 259-estimated 4,500 flies per trap) than in unbaited traps (72-109 flies per trap) (t-test, 13 d.f., $t = -4.56$, $P < 0.05$). For unbaited traps without CO_2 , catches of male *P. sergenti*, male *P. papatasi*, female flies (unfed and total females), and blood-fed female flies did not differ between traps with a metofluthrin device, a blank device, or no device (F-test, $P > 0.05$) (Table 2); thus, no effect of metofluthrin was noted. For suction traps baited with CO_2 , approximately 75% fewer *P. sergenti* males and blood-fed females (mean catches) were collected in traps with a metofluthrin device compared with a blank device ($F_{2,24} = 4.13$ and 4.62 , $P = 0.04$ and 0.03 , respectively); however, catches in traps with metofluthrin did not differ from catches in traps with CO_2 only (i.e., no device) (F-test, $P > 0.05$). For female flies, catches were barely not significant between treatments (ANOVA, $F_{2,24} = 3.1$, $P = 0.075$). For *P. papatasi* males, no differences were observed between treatments. None of the catches was significantly affected by day or trapping location.

Proportions of flies in CO_2 -baited traps were also compared among treatments. For female flies (unfed and total females), proportions were significantly higher in traps with a metofluthrin device ("CO₂ + MET") than in unbaited traps ("Trap only"), traps with a metofluthrin device ("MET"), and CO_2 -baited traps ("CO₂ only") (ANOVA, $F_{5,39} = 4.09$, $P < 0.05$; post-hoc Tukey HSD: $t = 0.61$ -0.884, $P < 0.05$). Conversely, the proportion of blood fed females was significantly higher in unbaited traps ("Trap only") than traps baited with CO_2 ("CO₂") (ANOVA, $F_{5,39} = 4.72$, $P < 0.05$; post-hoc Tamhane test: $t = 1.04$, $P < 0.05$); however, the addition of a metofluthrin device to baited or unbaited traps had no effect on catch proportions. The proportions of *P. papatasi* and *P. sergenti* males did not vary significantly between treatments (F-test, $P > 0.05$).

Regardless of whether CO_2 was present or absent, most of the flies inside the collection bags of traps paired with a metofluthrin device were dead during morning collections (Figure 4). A visual inspection of each device revealed approximately 20-40 unfed, dead flies on top of the metofluthrin cartridge. Presumably the flies got stuck on the cartridge after being sucked into the top of the device (Figure 1b). These flies were not included in the trap catches.

Sticky trap experiments

Approximately 20,400 flies were collected on 15 sticky

traps on two nights of trapping in October 2009. Overall catches on 7 October consisted of 41.6% female flies, and 11.0% of the females contained blood meals (Table 3). The proportion of female flies (i.e., catches of both unfed females and total females) did not differ from the proportion of male flies in sticky traps or the proportion of female flies in suction traps (F-test, $P > 0.05$). In addition, the proportion of blood-fed flies in sticky traps did not differ from the



Figure 2. Suction traps were operated in an updraft configuration and suspended in a vertical position by a tripod attached to rocks to prevent it from blowing over in strong winds. CO_2 from a thermos flask containing dry ice was vented at the trap entrance. A fan vaporizer device emanating metofluthrin was either placed directly below the trap entrance (shown) or clipped onto the side of the trap (not shown).



Figure 3. Sticky traps consisted of oiled plastic sheets placed on the ground and located approximately 2-3 m apart. The corners of each sheet were weighted with large stones to keep the sheets fixed in the wind. Sticky traps were baited with CO_2 from dry ice in a thermos flask (shown). For traps with a metofluthrin device or blank device, the device was placed at the center of the sheet next to the CO_2 outlet tube. The background shows the perimeter road extending to the village of Kfar Adummim in the Judean Desert, Israel.

Table 2. Mean (\pm SE) catches of sand flies (*Phlebotomus* spp.) in unbaited suction traps or traps baited with CO₂, and with or without a metofluthrin device ("MET") or blank device ("Blank"). Percentages represent the mean (\pm SE) of *P. papatasii* or *P. sergenti* males, females and blood fed females. Total catches include male and female flies representing all species.

Treatment	<i>P. papatasii</i> males (%) ^a	<i>P. sergenti</i> males (%) ^a	Females (%) ^b	Blood-fed females (%) ^c	Total				
Trap only	3.7 ± 1.6	(6.5 ± 2.8%)	48.5 ± 2.7	(93.5 ± 2.8%)	37.0 ± 3.7	(41.3 ± 1.9%)	2.7 ± 1.8	(14.1 ± 3.7%)	82.0 ± 2.9
Blank device	1.0 ± 0.6	(2.4 ± 1.3%)	40.3 ± 1.2	(97.6 ± 1.3%)	34.3 ± 5.4	(44.8 ± 3.4%)	6.0 ± 1.2	(10.9 ± 0.9%)	86.0 ± 7.7
MET	3.5 ± 1.4	(8.3 ± 4.1%)	49.7 ± 15.0	(91.7 ± 4.1%)	31.3 ± 9.6	(37.1 ± 1.4%)	4.8 ± 2.3	(15.9 ± 5%)	84.5 ± 25.4
CO ₂ only	21.3 ± 9.2	(5.0 ± 3.2%)	1,457.8 ± 308.8	(95.1 ± 3.3%)	1035.6 ± 213.3	(44.6 ± 3.9%)	52.7 ± 11.3	(5.3 ± 0.5%)	2,512.9 ± 516.3
CO ₂ + Blank	29.5 ± 11.8	(1.9 ± 0.7%)	2,155.2 ± 547.2	(98.4 ± 0.7%)	1522.5 ± 229.2	(44.1 ± 3.4%)	103.0 ± 30.6	(6.7 ± 1.2%)	3,706.7 ± 759.9
CO ₂ + MET	15.0 ± 6.7	(3.5 ± 1.2%)	502.9 ± 171.3	(96.5 ± 1.2%)	592.9 ± 122.3	(58.7 ± 4.5%)	28.9 ± 9.3	(4.9 ± 1.2%)	1,110.9 ± 292.8

^aPercentage of all male sand flies collected. ^bPercentage of all sand flies collected. ^cPercentage of all female sand flies collected.

Table 3. Mean (\pm SE) catches of sand flies (*Phlebotomus* spp.) in unbaited sticky traps or traps baited with CO₂, and with or without a metofluthrin device ("MET") or blank device ("Blank"). Percentages represent the mean (\pm SE) of *P. papatasii* or *P. sergenti* males, females and blood-fed females (7 October catches only). Flies collected in traps with CO₂ only are listed separately for 7 and 27 October. Total catches include male and female flies representing all species.

Treatment	<i>P. papatasii</i> males (%) ^a	<i>P. sergenti</i> males (%) ^a	No. females (%) ^b	No. blood fed (%) ^c	Total				
Trap only	9.3 ± 4.1	(15.7 ± 8.8%)	86.3 ± 32.4	(77.1 ± 6.3%)	43.7 ± 8.3	(40.5 ± 6.4%)	7.0 ± 0.6	(16.3 ± 1.8%)	110.3 ± 9.3
CO ₂ only (7 Oct)	32.0 ± 16.0	(2.6 ± 1.9%)	2,226.3 ± 856.0	(97.5 ± 1.9%)	1,608.7 ± 269.4	(45.8 ± 7.4%)	174.0 ± 97.0	(10.2 ± 4.6%)	3,866.7 ± 1,109.6
CO ₂ only (21 Oct)	—	—	—	—	—	—	—	—	1,135.3 ± 114.4
CO ₂ + Blank	—	—	—	—	—	—	—	—	982.0 ± 528.0
CO ₂ + MET	—	—	—	—	—	—	—	—	700.0 ± 72.1

^aPercentage of all male sand flies collected. ^bPercentage of all sand flies collected. ^cPercentage of all female sand flies collected.



Figure 4. Sand flies in collection cups for traps without a metofluthrin device (right) and with a device (left).

proportion of blood-fed flies in suction traps (F-test, $P > 0.05$). Overall catches of male flies consisted of 98.2% *P. sergenti* and 1.8% *P. papatasi* which was similar to the mean proportions in suction traps (t-test, 43 d.f., $t = -0.43$, $P > 0.05$).

Similar to suction trap catches, more flies were collected on sticky traps baited with CO₂ compared with unbaited traps (Table 3), especially on 7 October (range = approximately 1,800-5,600 total flies per trap) compared to 27 October (approximately 920-1,310 total flies per trap) (t-test, 8 d.f., $t = 28.0$, $P < 0.0001$). On 7 October, approximately 110 flies (range = 101-129 flies) were collected per unbaited trap compared with approximately 4,000 flies per CO₂-baited trap, a 35-fold difference. There were no differences in the proportions of *P. papatasi* males, *P. sergenti* males, unfed females, or blood-fed females between unbaited and CO₂-baited sticky traps (t-test, 4 d.f., $P > 0.05$) or between suction and sticky traps for either of the two treatments (t-test, 7 or 10 d.f., respectively, $P > 0.05$).

On 27 October, mean trap catches did not differ among the three treatments consisting of CO₂-baited traps paired with a metofluthrin device, blank device, or CO₂ only (ANOVA, $F_{2,9} = 0.48$, $P = 0.64$). However, the largest densities of flies were observed on the sheets located at either end of the linear arrangement of nine sticky sheets, which represented the “CO₂ only” and “CO₂ + Blank” treatments. Photographs of the traps showed sand flies were generally distributed normally across most of the sheet, except lower numbers in the northeast corner of each sheet (i.e., upwind side of sheet) (Figure 5). There were no discernable differences in the pattern of catches on sheets with or without a metofluthrin device or blank device. Similar to the results for suction traps, approximately 20-40 dead flies were observed on the metofluthrin cartridges in each device tested using sticky traps.

Climate conditions

Mean temperature decreased from 20-26° C at 18:00 to 18-20° C at 06:00. Mean wind speeds at a height of 2 m ranged from 0.2 to 8.0 m/s (median wind speed = 2.0 m/s).

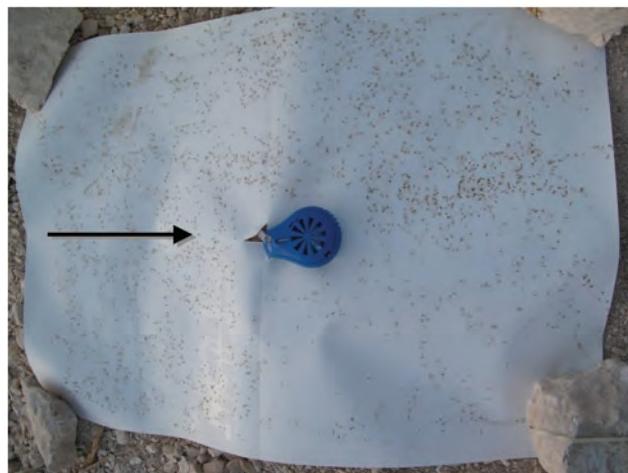


Figure 5. Typical distribution of sand flies on a sticky trap baited with CO₂ and a device. The arrow denotes the primary wind direction from the south.

At ground level, mean wind speeds ranged from 0 to 0.7 m/s (median = 0.2 m/s). Wind conditions were characterized by long periods of calm winds interspersed with short episodes of strong wind gusts, especially around sunset (5-7 October) and occasionally around 22:00 (21 and 27 October). The primary wind direction was from the east with variable winds between 22:00 and 02:00 on 6 and 7 October.

DISCUSSION

For catches from both suction and sticky traps (approximately 76,400 flies in total), male sand flies consisted of mostly *P. sergenti* (98.3%), which is consistent with proportions of *P. sergenti* in previous suction trap collections in Kfar Adummim (Schnur et al. 2004, Orshan et al. 2006, 2010). The only other species identified was *P. papatasi*, which was consistently found in traps in small numbers (1.7%). None of the male flies was identified as *P. tobii*, *P. syriacus*, or *P. alexandri* which have been sampled previously at the same study site (Schnur et al. 2004, Orshan et al. 2010). It is possible these species may have been present – albeit in very low numbers – in large trap collections from which small sub-samples of flies were selected for identification.

Female flies comprised 43% of the total catch, which is lower than the mean annual proportion of 56% described by Orshan et al. (2010). The overall proportions would be even lower (41%) if the disproportionately high proportion of unfed female flies in CO₂-baited suction traps with a metofluthrin device (58.7%) was omitted from the analysis. The relatively low proportions of females in our study might reflect the tendency of male flies to peak in October. The proportion of blood-fed females in catches of female flies (6.7% overall) was higher than the mean annual blood fed rate (3.5%) described by Orshan et al. (2010), which might also reflect differences in the proportion of blood-fed females at different times of the sand fly activity season.

Trials with suction traps showed the metofluthrin device did not reduce catches of flies when CO₂ was absent,

which suggests metofluthrin did not actively repel flies away from the trap. In addition, the blank device had no effect on catches in unbaited traps, which suggests the royal blue color of the device was not visually attractive regardless of the chemical. However, the observation that the majority of catches in all suction traps (CO_2 -baited or unbaited) with a metofluthrin device consisted of dead flies suggests metofluthrin exhibited significant insecticidal activity. This is supported by the observation that flies travelled close enough to the device to get sucked into its top by the action of the fan under the cartridge, whether the device was placed adjacent to a suction trap or in the middle of a sticky trap. It is unlikely that the flies that were sucked into the device significantly reduced the numbers of flies collected in the suction traps or on sticky traps. However, it is unknown whether sand flies entered blank devices, because the absence of a metofluthrin cartridge meant the flies would have been sucked through the fan and immediately expelled through the grill on the front of the device.

For trials with CO_2 -baited suction traps, catches of *P. sergenti* males were significantly higher in traps with a blank device but did not differ between traps with a metofluthrin device and CO_2 only. This result may be explained by the large variation in catches for traps with a blank device, an artifact of the trap placement, or variable wind conditions during each night of sampling. It is also possible that the insecticidal activity of metofluthrin affected sand flies that approached CO_2 -baited traps but died before they were drawn into the traps. Due to the occasional high wind gusts at the study location, it was not possible to place a (non-sticky) sheet under the trap entrance to determine whether flies approaching suction traps with a metofluthrin device were knocked down. Further studies are necessary to understand the behavior of sand flies approaching CO_2 sources in different concentrations of metofluthrin.

The low numbers of flies in suction traps with metofluthrin could have been due to the exclusion of flies that died from metofluthrin exposure before entering the trap, so repeating the evaluation using sticky traps allowed us to passively collect sand flies and hence differentiate between repellency and mortality regardless of prevailing wind conditions. The metofluthrin device did not affect catches in sticky traps, though the species and gender composition of the catches was not determined. For sampling with CO_2 -baited sticky traps (27 October), the larger densities of flies on sheets placed at both ends of the linear arrangement might be due to the short distance between sheets (3–5 m), the radius of attraction to CO_2 , and competition between traps. The wind direction was variable, particularly at low wind speeds, so the lower densities of flies at the northeast quadrant of the sheets (upwind side) might have reflected a tendency for the flies to land on the downwind end of the sheet as they approached the CO_2 outlet.

In conclusion, our results showed metofluthrin from the OFF! Clip-On fan vaporizer did not repel sand flies from entering suction or sticky traps in a field environment. Our results also support recent findings that metofluthrin is an effective volatile knockdown agent with insecticidal activity

(Lukwa and Chiwade 2008, Ishiwatari et al. 2009, Rapley et al. 2009). Controlled studies are needed to determine the relative effects of metofluthrin as a spatial repellent, contact irritant, and/or insecticide (Achée et al. 2009) against sand flies. In addition, future studies are necessary to determine the extent to which fan vaporizers emanating metofluthrin reduce sand fly bites, particularly if flies that acquire a sub-lethal dose to cause sufficient disorientation are still able to bite, as demonstrated by *Aedes aegypti* mosquitoes affected by metofluthrin from impregnated paper strips in a domestic environment (Rapley et al. 2009). However, the results of this study raise doubts regarding the ability of the metofluthrin fan vaporizer device to provide sufficient protection outdoors. The use of fan vaporizers that emanate volatile pyrethroids are likely to be much more effective indoors to prevent bites from endophilic sand fly vectors due to an accumulation of the active ingredient in a closed domestic environment, though indoor use raises separate issues of inhalation toxicity (EPA 2009), even at low concentrations. Further studies are warranted to determine the efficacy of fan vaporizer devices emanating higher concentrations of metofluthrin or other vapor active pyrethroids against sand flies. We also demonstrated that sticky traps provide a useful, passive method to screen potential area repellents in a field environment. Future studies that include more elaborate experimental designs in which compounds with demonstrated spatial repellency are placed around suction or sticky traps to determine their range of efficacy are warranted.

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